

## Quantitative Trait Loci Conferring Resistance to Fall Armyworm and Southwestern Corn Borer Leaf Feeding Damage

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### ABSTRACT

Southwestern corn borer (*Diatraea grandiosella* Dyar) and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] damage ratings were measured on an F2-derived maize (*Zea mays* L.) population segregating for leaf feeding resistance following artificial infestation with neonates. Damage ratings for each insect were recorded in replicated trials at three locations and used in conjunction with a genetic linkage map to identify quantitative trait loci (QTL) associated with resistance. Significant QTL and their interactions were estimated by multiple interval mapping analysis. Resistance to southwestern corn borer leaf feeding was fit to a model containing eight QTL and two interactions explaining 20% of the phenotypic variation. A model containing seven QTL and one interaction best fit resistance to fall armyworm leaf feeding damage, and it explained approximately 14% of the phenotypic variation. Three QTL located on chromosomes 6, 9, and 10 affect leaf feeding damage ratings of both insects with similar effects and gene action. Minor interaction effects were observed. The QTL on chromosomes 1, 5, and 9 correspond to previously identified regions affecting resistance to southwestern corn borer. Insect resistance genes including the *mir* family of genes located on chromosome 6 and the *glossy15* locus on chromosome 9 fall within chromosomal regions of QTL predicted in this study. This study confirms that resistance to fall armyworm and southwestern corn borer involves many of the same QTL and candidate genes for insect resistance include the *glossy15* candidate locus on chromosome 9.

**F**OLIAR DAMAGE to whorl stage maize plants by fall armyworm and southwestern corn borer can significantly reduce grain yield (Williams and Davis, 1984a). First generation larvae infest plants, causing vascular and leaf tissue damage leading to reduced yield potential. Literature documents a significant amount of research devoted to identifying resistant germplasm and identifying mechanisms of resistance to southwestern corn borer. Insect rearing and artificial infestation techniques have also been developed so that uniform selection pressure can be applied (Davis, 1997; Wiseman et al., 1980). In an attempt to address leaf damage by southwestern corn borer, genetic resistance has been identified, and germplasm lines have been developed (Williams and Davis, 1982, 1984b; Williams et al., 1990; CIMMYT, 1991).

There are several problems, however, with using germplasm lines in a breeding program to enhance southwest-

ern corn borer resistance. Few plant breeders have access to insect rearing facilities, which are necessary to maximize selection efficiency. Negative traits tend to mask genetic gains in resistance at early generations of backcrossing, and levels of resistance tend to be moderate. Quantitative trait mapping can be used to overcome some of these problems and facilitate manipulation of genetic resistance.

Through a series of separate studies, researchers have identified QTL associated with southwestern corn borer resistance in inbreds developed from Antigua Group 2 and Dominican Republic Group 1 germplasm. Resistance QTL ranging in number from five to nine were identified in tropical growing conditions accounting for between 32 and 52% of phenotypic variation in three mapping studies (Bohn et al., 1997; Groh et al., 1998; Khairallah et al., 1998). A majority of the QTL identified by Bohn et al. (1997) also conferred resistance to sugarcane borer (*Diatraea saccharalis* Fabricius). A comparison of these separate studies by Bohn et al. (2001) further noted the consistent identification of genetic regions on chromosomes 5 and 9, and that these regions tended to confer resistance to sugarcane borer as well. Willcox et al. (2002) later identified three QTL regions using the same resistant parent (CML67) and a different susceptible parent. Two of the three QTL coincided with earlier studies. In all studies, a QTL on chromosome 9 in or near bin 3 has been identified. The *glossy15* gene is located in this region and is a potential resistance gene for fall armyworm resistance.

In a study estimating combining ability for fall armyworm and southwestern corn borer resistance, Williams et al. (1989) observed a strong correlation of GCA for leaf feeding, larvae weights, and larvae number between the two pests. The authors suggested that selection for resistance to one insect pest could improve resistance to the other. This study was performed in two locations in Mississippi and included Mp704 as an inbred resistant to leaf feeding by southwestern corn borer and fall armyworm (Williams and Davis, 1982). Further studies suggested that vegetative phase change, which is controlled by the *glossy15* gene, is a primary mechanism affecting resistance to first generation fall armyworm and southwestern corn borer (Williams et al., 1998, 2000). Resistant maize lines completed the transition from the juvenile to adult vegetative stage earlier than susceptible lines. Larvae of both southwestern corn borer and fall armyworm exhibited reduced weight gain and produced less leaf damage on genotypes that moved from the juvenile to adult phase earlier. In addition, larvae fed lyophylized

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**Abbreviations:** CIM, composite interval mapping; LOD, log10-likelihood ratio; QTL, quantitative trait locus or loci; SSR, simple sequence repeat.

leaf tissue in artificial diet bioassays exhibited a strong correlation between larval weights and vegetative phase of leaves used in the diets (Williams et al., 2000). The *glossy15* locus has been demonstrated to control the onset of adult phase epidermal traits, specifically epicuticular waxes, leaf hairs, and cell wall composition (Moose and Sisco, 1994; Bergvinson et al., 1997). Plants containing the mutant *glossy15* gene exhibited earlier phase change to the adult vegetative state and increased resistance to European corn borer (*Ostrinia nubilalis* Hubn.) (Abedon et al., 1996).

Jiang et al. (1995) identified several cysteine proteinases expressed by genes designated *mir1* to *mir4* that accumulate in the whorl immediately following insect leaf feeding. Concentration of these proteinases was highly correlated with reduced larvae weight. Four of these genes are clustered on chromosome 6 bin 2 with single genes found on chromosomes 2, 9, and 10 (Pechen et al., 1999, 2000).

This study was conducted to map QTL conferring resistance to first generation fall armyworm and southwestern corn borer leaf feeding damage in the maize inbred Mp704 when expressed in a non-stiff stalk (Mo17) genetic background and grown in a semitropical environment. The QTL are compared with previously published loci for leaf feeding resistance to southwestern corn borer and QTL for resistance to fall armyworm are presented. In addition, interactions associated with QTL are measured and discussed and likely genetic models are proposed to account for genotypic variation of each trait.

## MATERIALS AND METHODS

A genetic mapping population consisting of 230 F<sub>2</sub>:3 families was created with maize inbreds Mp704 and Mo17 as the resistant and susceptible parents, respectively. Two experiments consisting of four replications each were conducted at Mississippi State, MS, in 2000, 2001, and 2002. Experiment 1 was infested with fall armyworm larvae and Exp. 2 with southwestern corn borer. Plots within replications were 5.1 m in length, thinned to 20 plants per plot and arranged in a randomized complete block design. Standard cultural practices were applied to experimental plots during each year.

Upon reaching the V7 stage of growth (Ritchie et al., 1986), Exp. 1 was infested with 30 fall armyworm larvae per plant and Exp. 2 was infested with 30 southwestern corn borer larvae per plant. In each experiment, neonate larvae were mixed with corn cob grits and placed in the plant whorl with a mechanical dispenser (Wiseman et al., 1980). Individual plants were rated for leaf feeding damage 14 d following infestation as described by Williams et al. (1989). Fall armyworm leaf feeding damage was visually rated using a scale of 0, no damage, to 9, many leaves destroyed. Southwestern corn borer leaf feeding damage was rated on a scale of 0, no visible leaf damage, to 9, long lesions on most leaves. Rating scales differ somewhat to accommodate the pattern of damage produced by each insect pest.

Individual plant ratings were used to calculate plot means for fall armyworm and southwestern corn borer damage. Data were combined from the three environments for each insect. An analysis of variance was performed using the general linear model procedure in SAS (SAS Institute, Cary, NC). Families were considered fixed effects and environments were considered random effects. Least-squares means of fall armyworm

and southwestern corn borer damage ratings were computed for each family across all environments.

The DNA was extracted from bulk tissue samples collected from approximately 20 plants of each F<sub>2</sub>:3 family as well as the parents and F<sub>1</sub>. Bulk tissue samples were frozen in liquid nitrogen and freeze dried before being ground. The DNA was extracted by the CTAB method (Saghai Maroof et al., 1984). Simple sequence repeat (SSR) markers obtained from the Maize Genetic and Genomics Database (<http://www.maizegdb.org/>; verified 19 July 2005) were used to genotype families. The SSR products were visualized on 4% (w/v) agarose gels. Markers that could not be resolved were viewed on high resolution, nondenaturing acrylamide gels (7%).

Linkage maps were determined by Carthagine mapping software with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.5 (Schiex and Gaspin, 1997). Composite interval mapping was performed to give an initial estimate of QTL for each insect damage rating by QTL Cartographer version 2.0 (Zeng, 1993, 1994; Basten et al., 1999). To estimate the genome-wide 0.05 significance threshold for QTL, 1000 permutations were performed within each trait data set (Doerge and Churchill, 1996; Doerge and Rebai, 1996). Five markers were used as cofactors and the forward regression method was selected to perform the analysis. Estimated QTL were used in a model to begin multiple interval mapping (Kao and Zeng, 1997; Kao et al., 1999). QTL search procedures were as follows: (i) search for significant QTL to add to model, (ii) re-estimate QTL positions, (iii) search for significant interactions, (iv) test significance of QTL/interactions in model and drop nonsignificant ones, (v) return to step one and repeat steps 1 to 5 until no additional QTL–interactions can be added to model, and (vi) optimize QTL positions and re-estimate model. Final models were selected on the basis of minimizing Bayesian information content criteria.

## RESULTS AND DISCUSSION

### Phenotypic Evaluation

Tests in Environment 1 were observed to have much higher leaf damage ratings than normal, possibly because of better larval survival, growth, and development after infestation. Mean leaf feeding ratings of parents were significantly different for both southwestern corn borer and fall armyworm, while the F<sub>1</sub> hybrid was not significantly different from Mp704 ( $P = 0.05$ ). Mean ratings for southwestern corn borer damage were Mp704 = 6.88, Mo17 = 7.98, and F<sub>1</sub> = 6.43. Mean ratings for fall armyworm damage were Mp704 = 6.95, Mo17 = 8.66, and F<sub>1</sub> = 7.14. The reduced level of differentiation between the two parents because of higher than normal damage ratings is not conducive to maximizing QTL identification and can mask loci with small effects. The F<sub>1</sub> hybrid also displayed resistance similar to Mp704, suggesting a dominant form of inheritance. However the increased plant vigor associated with the “hybrid effect” of the F<sub>1</sub> may account for this, especially in light of the predominantly additive QTL identified. Analysis of variance indicated a significant genotype  $\times$  environment interaction for southwestern corn borer resistance but not for fall armyworm resistance ( $P = 0.04$  and 0.99, respectively). To simplify analysis between the two traits, genotypic means were estimated across all environments for both damage ratings. Least squares means

**Table 1. Quantitative trait loci contributing resistance to leaf damage ratings by southwestern corn borer (SWCB) and fall armyworm (FAW).**

QTL	Trait	Location†	Effect(a)	Effect(d)	%Var‡
LDR1	SWCB	1.04	−0.1	−0.1	6
LDR2	FAW	1.09	−0.1	−0.2	13
LDR3	SWCB	1.11	−0.1	—	4.5
LDR4	FAW	2.08	0.1	0.25	9
LDR5	SWCB	5.02	−0.2	—	14
LDR6	SWCB/FAW	6.02	—	−0.2/−0.13	3/6
LDR7	SWCB	7.02	−0.1	0.5	23
LDR8	SWCB	7.03	—	−0.4	13
LDR9	FAW	7.04	−0.1	−0.3	12
LDR10	FAW	8.03	—	0.1	5
LDR11	SWCB/FAW	9.03	−0.3/−0.4	−/−0.1	15/36
LDR12	SWCB/FAW	10.04	−0.2/−0.1	−/−0.2	9/10
LDR4 × LDR12 FAW				0.5	6
LDR5 × LDR8 SWCB				0.2	3
LDR5 × LDR12 SWCB				−0.2	2

† Chromosome number and bin location.

‡ Percentage of genotypic variance explained by the QTL.

were calculated and used to represent phenotypes of mapping families.

### QTL Analysis

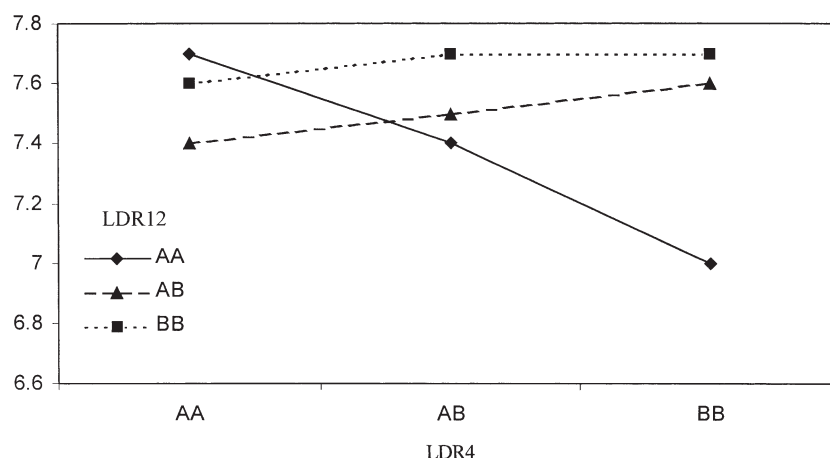
Initial composite interval analysis identified 10 and 5 QTL for southwestern corn borer and fall armyworm resistance, respectively. Estimated phenotypic variance explained by QTL ranged from less than 0.1 to 18% for resistance to southwestern corn borer and from less than 1 to 27% for resistance to fall armyworm. Primary QTL for resistance to southwestern corn borer were located on chromosomes 5 and 9 and for fall armyworm on chromosome 9. Initial models based on these results were input into multiple interval mapping analysis (MIM).

The final selected model for resistance to southwestern corn borer incorporated eight QTL and two interactions explaining approximately 20% of phenotypic variation (Table 1). QTL with greatest effect were located on chromosomes 5, 7, 9, and 10. Mp704 contributed the resistant allele in each case. Loci on chromosomes 6 and 7 displayed dominant gene action, while the rest were additive or intermediate. QTL closely linked on chromosome 7, designated LDR7 and LDR8, were acting in

repulsion and are closely linked. Epistatic interactions were minor, accounting for approximately 5% of the genotypic variation explained by the model.

Fall armyworm resistance was fit to a model containing seven QTL and one interaction that explained approximately 15% of phenotypic variation. Primary QTL were located on chromosomes 1, 7, 9, and 10. Mp704 was again responsible for contributing the resistance at five of the eight loci. Mo17 contributed to resistance at two minor QTLs on chromosomes 2 and 8 contributing 9 and 5%, respectively, to explained genotypic variation. Interactions between a QTL on chromosomes 2 (LDR4) and 10 (LDR12) exerted a significant negative effect on resistance (Fig. 1). The presence of Mo17 alleles at LDR4 reduced damage when LDR12 is homozygous for Mp704 while increasing damage in the presence of all other genotypes.

The QTL exhibiting pleiotrophic effects on leaf damage ratings of both insect pest were also observed. Loci on chromosomes 6, 9, and 10 appeared to affect leaf feeding damage ratings of both southwestern corn borer and fall armyworm, exhibit similar effects, and have similar gene action on both traits. The QTL for resistance to southwestern corn borer on chromosomes 1, 5, and 9 correspond to previously identified loci in related resistant germplasm (Bohn et al., 1997). Several of these QTL are located in genomic regions containing putative resistance genes. LDR6 located on chromosome 6 bin 2 corresponds to the location of the *mir* genes (*mir1*, *mir2*, *mir3c*, and *mir4*). This QTL from Mp704 reduced leaf feeding damage ratings from 0.13 to 0.15 points for both insects and displayed dominant gene action, similar to observed effects of *mir1* (Pechen et al., 1999). LDR11 located on chromosome 9 bin 3 has been consistently observed to contribute resistance to leaf feeding damage. This QTL reduced damage ratings by 0.25 to 0.3 points for both insects and expressed additive gene action. Fifteen percent of the genetic variation in the model was explained by LDR11 for southwestern corn borer resistance and 36% for fall armyworm resistance. The glossy15(*gl15*) locus, demonstrated to play a role in vegetative phase change from juvenile to adult phase



**Fig. 1. Interaction of two QTL (LDR4 and LDR12) affecting resistance to fall armyworm. Y-axis represents mean leaf damage rating. X-axis represents genotype at LDR4 where A = allele from Mp704 and B = allele from Mo17. Lines represent genotypic classes of LDR12.**



in maize leaves, is located in this region (Moose and Sisco, 1994). Williams et al. (1998) demonstrated that southwestern corn borer and fall armyworm resistant maize lines transitioned from juvenile to adult phase earlier than susceptible lines. The QTL including *gl15* has a similar effect on feeding damage suggesting that this gene plays a key role in conferring reduced feeding damage in a pleiotrophic manner to southwestern corn borer and fall armyworm. Other genes located within this large QTL interval may, however, be the true contributors to resistance. Further experiments comparing near-isogenic lines selected by markers from this population with and without the resistant allele and introgression of the null allele into the resistant parent are underway to further study the role *gl15* plays.

Multiple interval mapping greatly reduced the estimated amount of phenotypic variation explained by individual QTL when compared with CIM. This can be attributed to the increased precision of predicted QTL locations suggesting estimates from CIM are inflated. The MIM identified two closely linked QTL acting in repulsion that were missed on the basis of CIM. These loci as well as the interaction in Fig. 1 can realistically be managed by marker-assisted techniques, a task much more difficult to do by traditional means. Results also suggest that selection for resistance to both insect pests would be effective provided proper QTL are incorporated. Epistatic interactions are present, but most do not interfere significantly with primary QTL effects. The relatively small effect of genotypic  $\times$  environment interaction and repeated identification of QTL on chromosomes 6, 7, and 9 suggests that marker-assisted selection can be effectively performed at other locations without the need of insect rearing facilities. While further sources of resistance are needed to strengthen the program, this study provides a straightforward set of resistance genes to work with that are amenable to marker-assisted selection without additional equipment investment.

Further information related to this study including the detailed marker set, genetic map, and QTL mapping coordinates can be found at the Maize Genetic and Genomics Database (<http://www.maizegdb.org/>).

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## REFERENCES

- Abedon, B.G., P. Revilla, and W.F. Tracy. 1996. Vegetative phase change in sweet corn populations: Genetics and relationship with agronomic traits. *Maydica* 43:77–82.
- Basten, C.J., B.S. Weir, and Z.B. Zeng. 1999. QTL cartographer, version 1.13. Department of Statistics, North Carolina State University, Raleigh, NC.
- Bergvinson, D., J. Arnason, and J. Hamilton. 1997. Phytochemical changes during recurrent selection for resistance to the European corn borer. *Crop Sci.* 37:1567–1572.
- Bohn, M., M.M. Khairallah, C. Jiang, D. Gonzalez-de-Leon, D.A. Hoisington, H.F. Utz, J.A. Deutsch, D.C. Jewell, J.A. Mihm, and A.E. Melchinger. 1997. QTL mapping in tropical maize: II. Comparison of genomic regions for resistance to *Diatraea* spp.
- Bohn, M., S. Groh, M.M. Khairallah, D.A. Hoisington, H.F. Utz, and A.E. Melchinger. 2001. Re-evaluation of the prospects of marker-assisted selection for improving insect resistance against *Diatraea* spp. in tropical maize by cross validation and independent validation. *Theor. Appl. Genet.* 103:1059–1067.
- CIMMYT Maize Program. 1991. Announcement of CIMMYT inbred lines CML1 to CML139. Mexico City.
- Davis, F.M. 1997. Improved technologies for rearing lepidopterous pests for plant resistance research. In J.A. Mihn (ed.) *Insect resistant maize: Recent advances and utilization*, proceedings of an international symposium. p. 184–188.
- Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. *Genetics* 142:285–294.
- Doerge, R.W., and A. Rebai. 1996. Significance thresholds for QTL interval mapping tests. *Heredity* 76:459–464.
- Groh, S., D. Gonzalez-de-Leon, M.M. Khairallah, C. Jiang, D. Bergvinson, M. Bohn, D.A. Hoisington, and A.E. Melchinger. 1998. QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. *Crop Sci.* 38:1062–1072.
- Jiang, B., U. Siregar, K.O. Willeford, D.S. Luthe, and W.P. Williams. 1995. Association of a 33-Kilodalton cysteine proteinase found in corn callus with the inhibition of fall armyworm larvae growth. *Plant Physiol.* 108:1631–1640.
- Kao, C.-H., and Z.-B. Zeng. 1997. General formulas for obtaining the MLEs and the asymptotic variance-covariance matrix in mapping quantitative trait loci when using the EM algorithm. *Biometrics* 53: 653–665.
- Kao, C.-H., Z.-B. Zeng, and R.D. Teasdale. 1999. Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203–1216.
- Khairallah, M.M., M. Bohn, C. Jiang, J.A. Deutsch, D.C. Jewell, J.A. Mihm, A.E. Melchinger, D. Gonzalez-de-Leon, and D.A. Hoisington. 1998. Molecular mapping of QTL for southwestern corn borer resistance, plant height and flowering in tropical maize. *Plant Breed.* 117:309–319.
- Moose, S.P., and P.H. Sisco. 1994. Glossy15 controls the epidermal juvenile-to-adult phase transition in maize. *Plant Cell* 6:1343–1355.
- Pechan, T., B. Jiang, D. Steckler, L. Ye, L. Lin, D.S. Luthe, and W.P. Williams. 1999. Characterization of three distinct cDNA clones encoding cysteine proteinases from maize (*Zea mays* L.) callus. *Plant Mol. Biol.* 40:111–119.
- Pechan, T., L. Ye, Y.-M. Chang, A. Mitra, L. Lin, F.M. Davis, W.P. Williams, and D.S. Luthe. 2000. A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other Lepidoptera. *Plant Cell* 12: 1031–1040.
- Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1986. How a corn plant develops. Special report No. 48, Iowa State Univ. of Sci. and Tech. Coop. Ext. Serv., Ames, IA.
- Saghai Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014–8018.
- Schiex, T., and C. Gaspin. 1997. Cartagene: Constructing and joining maximum likelihood genetic maps. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* 5:258–267.
- Willcox, M.C., M.M. Khairallah, D. Bergvinson, J. Crossa, J.A. Deutsch, G.O. Edmeades, D. Gonzalez-de-Leon, C. Jiang, D.C. Jewell, J.A. Mihm, W.P. Williams, and D. Hoisington. 2002. Selection for resistance to southwestern corn borer using marker-assisted and conventional backcrossing. *Crop Sci.* 42:1516–1528.
- Williams, W.P., and F.M. Davis. 1982. Registration of Mp704 germplasm line of maize. *Crop Sci.* 22:1269–1270.
- Williams, W.P., and F.M. Davis. 1984a. Reaction of a resistant and a susceptible corn hybrid to various southwestern corn borer infestation levels. *Agron. J.* 76:855–856.
- Williams, W.P., and F.M. Davis. 1984b. Registration of Mp705, Mp706, and Mp707 germplasm lines of maize. *Crop Sci.* 24:1217.
- Williams, W.P., P.M. Buckley, and F.M. Davis. 1989. Combining ability

- for resistance in corn to fall armyworm and southwestern corn borer. *Crop Sci.* 29:913–915.
- Williams, W.P., F.M. Davis, and G.L. Windham. 1990. Registration of Mp708 germplasm line of maize. *Crop Sci.* 30:757.
- Williams, W.P., F.M. Davis, P.M. Buckley, P.A. Hedin, G.T. Baker, and D.S. Luthe. 1998. Factors associated with resistance to fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) in corn at different vegetative stages. *J. Econ. Entomol.* 91:1472–1480.
- Williams, W.P., P.M. Buckley, and F.M. Davis. 2000. Vegetative phase change in maize and its association with resistance to fall armyworm. *Maydica* 45:215–219.
- Wiseman, B.R., F.M. Davis, and J.E. Campbell. 1980. Mechanical infestation device used in fall armyworm plant resistance programs. *Fla. Entomol.* 63:425–432.
- Zeng, Z. 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA* 90:10972–10976 [Abstract].
- Zeng, Z. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457–1468.